**Congenital heart disease (CHD)** accounts for the largest percentage of morbid congenital abnormalities in the world, affecting approximately 8 out of every 1000 births [1]. CHD is thought to be caused by the disruption of a complex network of cardiac regulatory genes and proteins which together dictate proper heart formation [2]. The NKX2-5 gene, a master regulator of heart formation in vertebrates, encodes a homeobox transcription factor that interacts with several heart developmental factors [3]. Previous studies have shown that specific mutations in NKX2-5 lead to characteristic defects of CHD in humans, such as septal defects, arrhythmias, or eventual heart failure [4]. *Despite this detailed knowledge of how specific mutations in NKX2-5 give rise to CHD defects, the role of NKX2-5 in cardiac morphogenesis and dysmorphogenesis remains poorly understood*.

My **primary goal** is to uncover the function of NKX2-5 in heart formation and CHD. Mice have highly similar genetic and physiological characteristics to those of humans, a short life span, and a high breeding rate [5]. For these primary reasons, among others, mice will be used as the organism to model CHD found in humans. I **hypothesize** that conserved mutations between mice and humans disrupt the function of NKX2-5 and its protein-protein interaction network, leading to CHD. My **long-term goal** is to determine the mechanism by which specific mutations of NKX2-5 give rise to specific defects of CHD. Such an insight would offer novel opportunities to develop cures or treatments to certain, if not all, defects of CHD.

**Aim 1: Determine what amino acids are important for NKX2-5 function and heart formation.**

**Approach:** ClustalOmega analysis will be performed to identify conserved NKX2-5 amino acids between mice and humans. Then, CRISPR/Cas9 will be used in mice to induce mutations that have been shown to cause CHD in humans. Mutant mice will be screened for CHD.

**Hypothesis:** I hypothesize that mutations in conserved amino acids of mice will result in similar phenotypic effects in humans.

**Rationale:** The presence of conserved amino acids is crucial for the function of NKX2-5 and ultimately, proper heart formation across homologous species.

**Aim 2:** **Determine the effect of conserved mutations on the transcription of cardiac-specific proteins and CHD.**

**Approach**: STRING will be used to identify protein-protein interactions of NKX2-5 in mice and humans.Mice with CHD phenotypes will be sacrificed from Aim 1. RNA-Sequencing will be performed on the defective hearts of these mice.

**Hypothesis**: There will be significant changes in the transcription levels of NKX2-5 and its interacting proteins.

**Rationale**: NKX2-5 both positively and negatively regulates numerous genes during heart formation. Mutations in NKX2-5 can result in ectopic levels of downstream interacting proteins, leading to CHD.

**Aim 3: Identify the effect of conserved mutations on protein-protein interactions of NKX2-5.**

**Approach:** CRISPR/Cas9 will be used in mice to induce conserved mutations that were found to cause CHD phenotypes in Aim 1. After fetal development, the mutant mice will be sacrificed and far-western blots and co-immunoprecipitation will be performed to investigate the NKX2-5 protein-protein interaction network of mutant mouse hearts.

**Hypothesis:** The conserved mutations will result in a decrease in the interaction of NKX2-5 with known protein-protein interactors.

**Rationale:** The conserved amino acids in NKX2-5 are essential for NKX2-5 to bind to or be bound by known interacting proteins.

**References**

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